

# PCR protocol

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RBRC 03116

Strain name C57BL/6J-Tg(CAG-SCAT3)43Miur

| Primer | primer_name | primer_seq                | length |                         |
|--------|-------------|---------------------------|--------|-------------------------|
| 1      |             | CAGAAGCTGATCTCGGAGGA      | 20     | Depositor's primer      |
| 2      |             | GCACTGCACGCCCCAGGT        | 18     | Depositor's primer      |
| 3      | oIMR0042    | CTAGGCCACAGAATTGAAAGATCT  | 24     | JAX Genotyping Protocol |
| 4      | oIMR0043    | GTAGGTGGAAATTCTAGCATCATCC | 25     | JAX Genotyping Protocol |
|        |             |                           |        |                         |
|        |             |                           |        |                         |
|        |             |                           |        |                         |
|        |             |                           |        |                         |
|        |             |                           |        |                         |
|        |             |                           |        |                         |

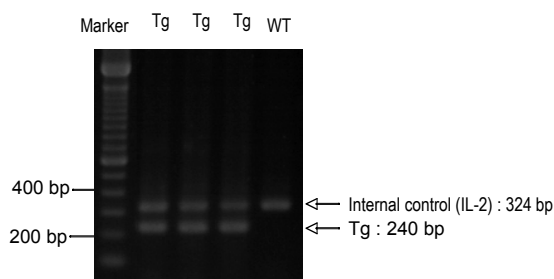
## Reaction Components

|        |                                 |
|--------|---------------------------------|
| Enzyme | TaKaRa Ex Taq (Cat. No. RR001A) |
|--------|---------------------------------|

## Reaction Conditions

|            | Conc             | Run 1 | Run 2 | Run 3 | Run 4 |
|------------|------------------|-------|-------|-------|-------|
| H2O        |                  | 15.3  |       |       |       |
| PCR buffer | 10 X             | 2.5   |       |       |       |
| dNTP       | 2.5 mM           | 2.0   |       |       |       |
| primer 1   | 10 pmol/ $\mu$ l | 1.0   |       |       |       |
| primer 2   | 10 pmol/ $\mu$ l | 1.0   |       |       |       |
| primer 3   | 10 pmol/ $\mu$ l | 1.0   |       |       |       |
| primer 4   | 10 pmol/ $\mu$ l | 1.0   |       |       |       |
| DNA        |                  | 1.0   |       |       |       |
| Taq        |                  | 0.2   |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
|            |                  |       |       |       |       |
| total      |                  | 25    |       |       |       |

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 94        | 180        |                       |
| 2    | 94        | 30*        |                       |
| 3    | 55        | 30*        |                       |
| 4    | 72        | 60*        | *Repeat for 30 cycles |
| 5    | 72        | 300        |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |



## Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

## Gel

2% agarose in 1 X TAE (100 V, 30 min)

## PCR products

| primer_set |          | product_size | product                 |
|------------|----------|--------------|-------------------------|
| primer 1   | primer 2 | 240 bp       | Tg                      |
| primer 3   | primer 4 | 324 bp       | Internal control (IL-2) |
|            |          |              |                         |
|            |          |              |                         |
|            |          |              |                         |

Comment